Biotransformation of the potato stress metabolite, solavetivone, by cell suspension cultures of two solanaceous and three non-solanaceous species

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Received May 15, 1984/Revised version received July 31, 1984 – Communicated by A. R. Gould

ABSTRACT

Cell suspension cultures of several plant species which were exogenously supplied solavetivone accumulated four hydroxylated derivatives. Only solanaceous cell cultures converted exogenously supplied solavetivone to sesquiterpenoid compounds identified with the plant response to biotic stress. Failure of potato cell cultures to make these compounds under biotic stress may be linked to their inability to produce adequate levels of solavetivone.

ABBREVIATIONS: Murashige and Skoog (1962), MS; 2,4-dichlorophenoxyacetic acid, 2,4-D; Tobacco Mosaic Virus, TMV; 1-naphthaleneacetic acid, NAA; thin layer chromatography, TLC; gas chromatography, GC; gas chromotography/mass spectrometry, GC/MS; nuclear magnetic resonance, NMR.

INTRODUCTION

In the interaction of potato tubers (Solanum tuberosum) and Phytophthora infestans or cell free mycelial homogenates of the fungus (fungal elicitor), solavetivone (katahdinone), a vetispirane sesquiterpene, is produced (Coxon et al. 1974). Kalan and Osman (1976) reported that solavetivone was converted to isolubimin when applied to the surface of potato slices. A pathway was also proposed wherein solavetivone was transformed to the norsesquiterpene rishitin via isolubimin, lubimin, and hydroxylubimin. Masamune et al. (1978) proposed an almost identical pathway for which confirming evidence was offered (Murai et al. 1982) and suggested that solavetivone serves as an enzyme activator for the formation of stress metabolites from acetic acid.

In our experience when callus or suspension cell cultures of S. tuberosum are challenged by the fungus or fungal elicitor, stress metabolites of the above pathway are not routinely or predictably produced. Erratically, lubimin, hydroxylubimin, and rishitin were observed following the interaction. Since we have never observed solavetivone in potato callus or cell suspensions, it was hypothesized that the cultures were incapable of producing adequate levels of this intermediate to sustain the pathway, or that solavetivone may be rapidly converted to pathway metabolites or may lead to other metabolites not in the proposed scheme.

In the present study, solavetivone was incorporated into potato suspension cell cultures to test the above hypotheses. Since Alves et al. (1979) had reported enhanced stress metabolite formation in potato tuber slices with an atmosphere of ethylene in air, such an atmosphere was included in this study. The fate of solavetivone also was determined when exposed to suspension cell cultures of tobacco and of non-solanaceous plants which do not produce the compound.

MATERIALS AND METHODS

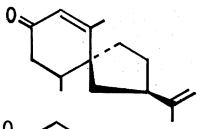
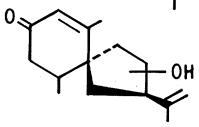
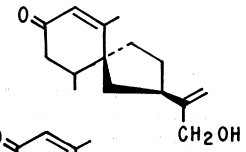
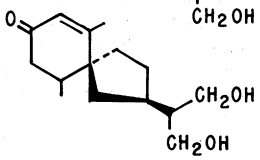
Plant Cell Cultures

Callus of S. tuberosum cv. Kennebec was initiated from tuber tissue on a modified solid MS medium containing 6 mg/l NAA, 2 mg/l 2,4-D, and 0.21 mg/l kinetin. Friable calluses were transferred to IB5C (Gamborg and Wetter 1975) liquid medium and incubated at 27°C on a rotary shaker at 150 oscillations/min. The resulting cell suspensions were maintained by transferring 20 ml of cell suspension into 40 ml fresh IB5C in 250 ml flasks at 5-6 day intervals. Callus of Glycine max (L) Merr. cv. Mandarin was initiated from epicotyl explants and a suspension culture developed as described by Fett and Zacharius (1982). This was maintained on IB5 in a manner similar to that for S. tuberosum. A suspension culture of Papaver somniferum obtained from A-F. Hsu of this laboratory was maintained as described by Hsu (1981). A suspension culture of Rosa sp (Paul's Scarlet Rose) was obtained from A. Marcus (Inst. for Cancer Research, Phila., PA 19111) and was grown and maintained by subculturing every 21 days as described by Ramagopal et al. (1977). Suspension culture of Nicotiana tabacum cv. bright yellow was obtained from L. Owens, ARS, Beltsville, MD 20705, and grown and maintained by subculturing every 3 days on a medium described by Uchimiya and Murashige (1974).

Interaction, Extraction, and Analysis

Solavetivone was isolated and purified from potato tubers infected with P. infestans with silica gel columns and silica gel TLC. The compound was supplied to the suspension cell cultures by addition and dispersion in the cell media with a sonifier at the level of 0.5 or 1.0-1.2 mg/10 ml of cell culture. Cells with added solavetivone were incubated in air at 27°C with constant shaking at 150 oscillations/min. Potato suspension cultures were maintained in an air

Table 1. Conversion products of solavetivone by plant suspension cultures

Metabolite	Structure	Parent ion	R_f [a]	Retention time (min) [b]
Solavetivone		218	0.80	13.5
A [c]		234	0.53	24.0
A'	Epimer of A?	234	0.58	24.0
B		234	0.34	29.2
C [d]		252	0.07	33.0

[a] TLC on silica gel developed with cyclohexane:ethylacetate (1:1).

[b] GC with 3% OV-225 on gas-chrome Q (100-200 mesh, 1.8 m X 2 mm ID glass column), temperature programmed at 4°C/min.

[c] Insufficient NMR data does not permit the unequivocal assignment of the ring hydroxyl.

[d] Tentative; insufficient NMR data does not permit the unequivocal assignment of the isopropenyl hydroxyls.

atmosphere and in an atmosphere consisting of 10 ppm ethylene in air. At intervals 10 ml aliquots of cells and media were withdrawn, and as one, were extracted four times, each with an equal volume of $CHCl_3$. The extracts were combined and solvent removed at room temperature under a stream of N_2.

Components of the residue, separated on Silica gel G TLC plates with a cyclohexane:ethyl acetate (1:1) solvent system, were visualized by spraying with $CHCl_3$ saturated $SbCl_3$ reagent and heating at 60°C for 5 min. Larger quantities of the products resulting from the potato culture incubation were obtained by preparative TLC. Components on the TLC plates were divided into 14 fractions representing as many visible spots. Fractions were eluted with ethyl acetate and rechromatographed until a single component was evident on GC (Heisler et al. 1978).

Structural identification of well-described compounds and tentative characterization of unknown metabolites was made on the basis of GC/MS and NMR spectra data obtained on a Hewlett Packard[2] GC/MS Model #5992B and a Jeol NMR Model FX60Q.

[2] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

RESULTS AND DISCUSSION

When suspension cell cultures of each of the plant species were exposed to solavetivone for several hours or more, the level of this compound decreased with a concomitant appearance of metabolites A, B, and C (Table 1); metabolite A' also appeared in the soy and potato cultures. Incubation/shaking of solavetivone in IB5 or IB5C media without plant cells did not result in the production of any A, A', B, or C. Mixtures on TLC and GC demonstrated that A and B from soy, poppy, and rose cultures were identical. Homogenates of soy and poppy cell suspensions incubated with solavetivone failed to produce any of the metabolites found with intact cell suspensions.

Metabolites A, A', B, and C were characterized by GC/MS and NMR yielding the data and tentative structures shown in Table 1.

A decrease of solavetivone was noted within 7 h but never fully disappeared, while metabolite A, the major product of the soy cultures appeared at 7 h and increased until the experiment was terminated (144 h). Of 21 mg solavetivone supplied to the culture, 8 mg A was isolated. B and C arose between 72-144 h incubation, each representing 2% of the initial sesquiterpene supplied the culture.

Table 2. Solavetivone exposed to N. tabacum cultures for 70 h

Compound	µg/10 ml
Residual solavetivone[a]	524
Rishitin	252
Lubimin	198
A	40[b]
A'	Tr
B	60[b]
C	29[b]
Total	1134

[a]Initial concentration 1200 µg/10 ml of cell suspension.

[b]Estimated values based on a lubimin standard.

Exposure of solavetivone to suspension cells of poppy resulted in little residual compound after 18 h and none by 72 h; the major conversion product was B and a small amount of A. Paul's Scarlet rose produced the same hydroxylated derivatives found in soy and poppy cultures with B the major compound.

The potato culture incubated with solavetivone, with or without ethylene present, yielded the same level of products. Separation of the 14 $SbCl_3$-positive areas on TLC, revealed the following components. Lubimin was isolated and demonstrated with GC/MS to be identical with authentic lubimin. Lubimin in cultures was evident at 92 h, increased to 144 h, and then declined. Isolubimin, barely detected at 5 h, increased in concentration to 44 h, and disappeared by 92 h. Trace amounts of rishitin—usually the main phytoalexin in tuber slices—were found at 92 h with or without an ethylene atmosphere. Hence, exogenous ethylene was not a requirement for and did not enhance stress metabolite production in this potato cell culture. A, B, and C were present in low amounts over the entire time course of the study.

The tobacco culture, after 70 h incubation with solavetivone, was found to contain large amounts of lubimin and rishitin, residual solavetivone, and small amounts of A, A', B, and C. The product distribution is shown in Table 2.

Fujimori et al. (1977) reported finding solavetivone in air-cured leaves of N. tabacum, and it subsequently was reported in N. tabacum infected with TMV (Fujimori et al. 1979; Ito et al. 1979). Uegaki et al. (1981) found 3-hydroxy solavetivone in TMV stressed leaves of N. tabacum. However, rishitin, lubimin, and hydroxylubimin have not been seen in leaves of stressed Nicotiana sp. Budde and Helgeson (1981) have identified rishitin and capsidiol in tobacco callus challenged with Phytophthora parasitica var nicotianae.

When provided solavetivone, only the solanaceous species described here were capable of producing compounds associated with the potato tuber hypersensitive response and additionally, small amounts of A, A', B, and C. Cell cultures of non-solanaceous species which are incompetent of the synthesis of sesquiterpernoid pathway compounds utilizing solavetivone, can only oxygenate/hydroxylate the exogenously supplied vetispirane structure.

Of particular interest is the finding of Anderson et al. (1977) of four vetispirane sesquiterpene glycosides in flue-cured tobacco, three of which, on treatment with β-glycosidase, yielded three aglycones related to solavetivone with a conspicuous similarity to the three compounds derived from plant cell suspensions incubated with solavetivone. Metabolites A, B, and C are consistent with the aglycone structures of Anderson et al. The observations with cell cultures suggest that the aglycones isolated from tobacco leaf glycosides resulted from enzymatic action common to a diverse number of plant species and are not unique to N. tabacum. The aglycone precursor, however, is unique to solanaceous species. It also may be that glycosylation of the solavetivone derivatives by the tobacco plant is a stress response to flue-curing which has an analogy to bacterial stress of soybean leaves (Osman and Fett 1983), fungal stress of alfalfa leaves (Olah and Sherwood 1970), and the accumulation of coumarin glycosides on bacterial attack of solanaceous hosts (Sequeria 1969).

Potato cell cultures which do not produce the sesquiterpene compounds in response to biotic stress are able to transform exogenously supplied solavetivone to those pathway metabolites, or conceivably solavetivone acts as an activator (Murai et al. 1982) in their de novo synthesis. Since solavetivone has not been detected in these cultures, a block appears to occur at this point on the pathway.

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